CLAIMS

1. Improved method for detecting specific target cells in cell suspensions of mixed cell populations and in fluid systems containing mixed cell populations, and in single cell suspensions prepared from solid tissues, characterized by comprising the following steps:

1.1. coating, by a per ce known procedure, paramagnetic particles or beads with either, a) antibodies, or antibody fragments directed against membrane structures specifically expressed on target-cells and not on non-target-cells in the cell mixture or;
b) antibodies, preferably polyclonal anti-mouse or monoclonal rat anti-mouse antibodies or anti-human antibodies, capable of binding to the Fc-portions of the said antibodies, directed against the membrane structures; and

1.2.1. mixing the target-cell-associating antibodies (murine or human) which is attached to the said particles or beads, or attached to the beads pre-coated with anti-mouse or antihuman antibodies recognizing the Fc-portions of the target-associating antibodies, with the cell suspension containing the target-cells, or,

1.2.2. mixing free target-cell-associating antibodies with the cell suspension containing the target cells and incubate this mixture for 5-10 min to 2 h, preferably 30 min, at a temperature between 0°C and 20°C, preferably 4°C under gentle rotation, and;

1.3. incubating the mixture of the cell suspension and target-associating antibodies attached to paramagnetic particles or beads (1.2.1), or paramagnetic particles or beads, precoated with anti-mouse or anti-human antibodies recognizing the Fc-portion of the target-associating antibodies, to the mixture of incubated free target associating antibody and cell suspension (1.2.2.), and incubating, for 5-10 min to 2 h,

preferably 30 min, at a temperature between 0°C and 25°C, preferably 4°C, under gentle rotation, and;

1.4.1. if the target cell population is contained in blood or bone marrow aspirates the hydrophobic forces associated with antibody-coated particles are reduced by pre-incubating the antibody-coated particles and the cell suspension with mild detergents in suitable concentrations, e.g. Tween 20 in concentrations less than 0.1% for 30 min at 4°C, and/or

1.4.2. by incubating the cell suspensions, untreated or pretreated with formalin, alcohol or other fixatives, with other antibodies or antibody fragments binding to extracellular or intracellular molecules present in the target cells and the antibodies used are labeled in advance by peroxidase, alkaline phosphatase, or other enzymes permitting visualization of the binding by addition and incubation with relevant substrates, or

1.4.3. the antibody fragments are biotinylated and the binding visualized when adding the incubating with avidin complexed to peroxidase, alkaline phosphatase, or other enzymes, with addition and incubation with relevant substrates, or

1.5.1. subjecting the incubated paramagnetic particle-antibodies-cell mixture (1.3) to a magnetic field if the density of target-cells is low, or if the ratio of target cell/total cells in the cell mixture is low (≤ 1 %) and then examining and counting the stained or unstained particle-target-cell complexes in the cell suspension, using a micropscope and/or a suitable cell/particle counting device, or,

1.5.2. examining and counting the target-cells in the incubated mixture of paramagnetic particles, antibodies and cell mixture (1.3), or in the case when the antibodies or antibody fragments are conjugated to non-paramagnetic particles that can be visualized directly because of colour or through enzymatic activation, using a microscope and/or a suitable cell/particle

counting device if the ratio of target-cells/total cells in the cell suspension is adequate (> 1 %).

2. Method according to claim 1, characterized by directing the antibody or fragments thereof against the antigens in normal, living cells, such as liver hepatocytes, Kupffer cells and endothelial cells type 1 and 2 and Clara cells of the lung, endothelial cells of specific organs, pancreatic exocrine and endocrine cells, kidney tubule cells, bladder epithelial cells, brain glial and ependymal cells, bladder and prostate epithelial cells, ciliated cells of airways, different subpopulations of mucosal cells in the gastrointestinal tract, pituitary cells, and other endocrine cells in various hormone-producing organs.

3. Method according to claim 2, characterized by using as the said target-cell antibody an antibody which is reactive with antigens present on subpopulations of normal cells and oncogenic products expressed on the membrane of normal tissue cells.

4. Method according to claim 3, characterized by using as the said positive selecting antibody, an antibody which is directed against growth factor receptors on the membrane of normal cells, for example the EGF-receptor, PDGF (A and B) receptor, insuline receptors, insuline-like receptors transferrin receptor, NGF and FGF receptors.

5. Method according to claim 4, characterized by using an antibody directed against the group of integrins and other adhesion membrane molecules, and MDR proteins in normal cells.

6. Method according to claim 5, characterized by directing the antibody or fragments thereof against antigen or receptors in cells with abnormal developmental patterns, preferably such as primary and metastatic cancer cells.

claim 6

7. Method according to ~~one of the preceding claims~~, characterized by using as the said target-cell associating antibodies, antibodies of the IgG isotype, or $F(ab')_2$ or F(ab) fragments, or IgM, or fragments of IgM.

claim 7

8. Method according to ~~one of the preceding claims~~, characterized by preparing the mentioned cell suspension from mixed cell populations comprising mammalian tissues, for examples human bone marrow and peripheral blood, from pleural and peritoneal effusions, other body fluids, for example urine, cerebrospinal fluid, semen, lymph, or from solid tumors in normal tissues and organs, for example liver, lymphatic nodes, spleen, lung, pancreas, bone tissue, central nervous system, prostatic gland, skin and mucous membranes.

claim 8

9. Method according to ~~one of the preceding claims~~, characterized by that the antibody or antibody fragments is directed against groups of antigen determinants, such as those listed in the Table 1 of the specification.

claim 9

10. Method according to ~~one of the preceding claims~~, characterized by using as the said target-cell antibody an antibody or antibody fragment which is directed against growth factor receptors and oncogene products expressed on the membrane of malignant cells, for example insuline receptors, insuline-like receptors and FGF receptors in addition to those listed in Table 1 of the specification.

claim 10

11. Method according to ~~one of the preceding claims~~, characterized by using an antibody or antibody fragment directed against the group of integrins, other adhesion membrane molecules and MDR proteins in abnormal cells as listed in Table 1.

claim 11

12. Method according to ~~one of the preceding claims~~, characterized in that the used antibodies, antibody fragments

or combinations of these are directed to the antigen determinants as listed in Table 1 of the specification.

13. Method according to claim 12 ~~one of the preceding claims~~, characterized by using as the said antibody an antibody which is reactive with antigens present on abnormal cells, for example breast, ovarian and lung carcinoma cells, melanoma, sarcoma, glioblastoma and cancer cells of the gastrointestinal and genitourinary tract, and of the reticuloendothelial system, and/or target-cells associated with non-neoplastic diseases, such as cardiovascular, neurological, pulmonary, autoimmune gastrointestinal, genitourinary, reticuloendothelial and other disorders.

14. Use of the detection method according to claim 13 ~~one of the preceding claims~~, for isolation of target-cells, whereby the complex of cells and the paramagnetic particles are exposed to a magnetic field and the resulting magnetically aggregated cells are further subjected to biological, biochemical and immunological examinations, including also characterisation of specific genes at the DNA, mRNA and protein level, including polymerase chain reaction (PCR) and reverse transcriptase PCR.

15. Use of the method for detection of specific target-cells according to claim 14 ~~one of the preceding claims~~, whereby it is established in vitro cell cultures from the separated paramagnetic particle-target-cell-complexes, and/or for inoculation into immunodeficient animals, preferably to establish human tumor xenografts in the said animals.

16. Kit for performing the method according to claim 15 ~~one of the preceding claims~~, characterized by that it comprises;
1, specific antibodies or antibody fragments directed to the antigen receptors on the wanted target-cells, where said antibody or antibody fragment is bound or can be bound to included paramagnetic particles, without removing their antigen-binding ability, and/or

2, paramagnetic particles precoated with specific anti-Fc antibodies, preferably polyclonal anti-mouse, or monoclonal rat anti-mouse, or anti-human antibodies, capable of binding to the Fc portions of the target-cell associating antibodies, and specific free target-cell antibodies, and/or

3, paramagnetic particles precoated with specific anti-Fc antibodies, preferably polyclonal anti-mouse, or monoclonal rat anti-mouse, or anti-human antibodies, capable of binding to the Fc-portions of the target-cell associating antibodies, bound to specific anti-target-cell antibodies, and/or

4, other specific antibodies or antibody fragments directed against antigens/receptors within or on the wanted target cells, where said antibodies or antibody fragments are conjugated to biotin, peroxidase, alkaline phosphatase, or other enzymes, or where said antibodies or antibody fragments are bound to non-paramagnetic particles with specific colours or with bound enzymes such as peroxidase and alkaline phosphatase.

add C

add D'

add E'

add G

add I22